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Milk fatty acid profile from grazing buffaloes fed a blend of soybean and linseed oils

[*Perfil de ácidos graxos do leite de búfalas a pasto recebendo uma mistura de óleo de soja e linhaça na dieta*]

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ABSTRACT

The aim of the study was to examine the changes in milk fatty acid (FA) profile of grazing buffaloes fed either low (L, 276g/d) or high (H, 572g/d) doses of a blend (70:30, wt/wt) of soybean and linseed oils. Fourteen multiparous Mediterranean buffaloes grazing on a native pasture were fed 4 kg/day of a commercial concentrate containing no supplemental oil over a pre-experimental period of ten days. The baseline milk production and composition and milk FA profile were measured over the last three days. After this pre-experimental period the animals received the same concentrate added with either the L or H oil doses for 26 additional days. Milk yield (g/animal/day) did not differ at the start (1776 ± 522 and 1662 ± 291 for L and H, respectively, P<0.622) or at the end of the trial (4590 ± 991 and 4847 ± 447 in L and H, respectively, P<0.543). Baseline milk fat content (g/kg) averaged 77.1 (±20.5) in L and 74.3 (±9.9) in H (P<0.10) and was reduced (P<0.031) to 60.7 (±23.6) and 49.4 (±11.2) (P<0.0031) respectively after L and H with no differences between treatments (P<0.277). Baseline milk protein content (L=43.2 ± 3.4 and H= 44.3 ± 6.9g/kg) increased after oil supplementation (P<0.0001) in both L (73.2 ± 6.0g/kg) and H (68.4 ± 4.9g/kg) without differences between oil doses (P<0.123). Milk fat content of 14:0 decreased after oil supplementation only in the H treatment (5.29 to 4.03, P<0.007) whereas that of 16:0 was reduced (P<0.001) at both L (24.49 to 19.75g/100g FA) and H (25.92 to 19.17g/100g FA) doses. The reduction of total content of 12:0 to 16:0 was higher (P<0.052) in H (32.02 to 23.93g/100g FA) than L (30.17 to 25.45g/100g FA). Vaccenic acid content increased (P<0.001) from 5.70 to 13.24g/100g FA in L and from 5.25 to 16.77 in H, with higher results in the in H treatment (P<0.001). Baseline rumenic acid was sharply increased (P<0.001) in L (1.80 to 4.09g/100g FA, +127%) and H (1.60 to 4.61g/100g FA, +187%) with no differences between L and H (P<0.19). Overall, these results indicate a pronounced improvement in the nutritional value of milk fat from grazing buffaloes fed little amounts (0.276g/day) of a blend of soybean and linseed oils.

Keywords: buffaloes, conjugated linoleic acid, lactation, soybean oil, linseed oil

RESUMO

O objetivo do presente estudo foi avaliar as mudanças no perfil de ácidos graxos do leite de búfalas leiteiras recebendo baixas (B, 276g/d) ou altas (A, 572g/d) doses de uma mistura de óleos de soja e linhaça (70:30, peso/peso) na dieta. Quatorze búfalas multíparas da raça Mediterrânea, mantidas em pastagens nativas, receberam 4kg/dia de um concentrado comercial sem adição de óleo (pré-tratamento) ao longo de um período pré-experimental de 10 dias. A produção de leite individual e amostras de leite foram coletadas individualmente para determinação dos valores basais de composição e perfil de ácidos graxos do leite nos últimos três dias. Após este período, os animais receberam o mesmo concentrado adicionado de B ou A por 26 dias. A produção de leite (g/animal/dia) não diferiu no início (1776 ± 522 e 1662 ± 291 para B e A, respectivamente (P<0,622) e no final do período experimental (4590 ± 991 e 4847 ± 447 para L e H, respectivamente, P<0,543). O teor de gordura do leite (g/100g) apresentou valores médios de 77,1(± 20,5) para B e 74,3 (±9,9) para A (P<0,10) durante o período pré-tratamento, mas foi reduzido (P<0,03) após o

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fornecimento das dietas com óleo para 60,7 (± 23,6) e 49,4 (± 11,2), respectivamente para B e A, não havendo diferenças entre tratamentos (P<0,277). Os teores basais de proteína do leite (B=43,2 ± 3,4 e A=44,3 ± 6,9g/kg) aumentaram após a suplementação com óleo (P<0,0001) em ambos B (73,2 ± 6,0g/kg) e A (68,4 ± 4,9g/kg), não ocorrendo diferenças entre tratamentos (P<0,123). O teor médio basal de 14:0 na gordura do leite (4,76g/100g AG) foi reduzido após a suplementação da dieta com óleo somente no tratamento A (5,29 para 4,03, P<0,007). O teor de 16:0 na gordura do leite foi reduzido (P<0,001) nos tratamentos B (24,49 para 19,75g/100g AG) e A (25,92 para 19,17g/100g AG). A redução nos teores de 12:0+14:0+16:0 na gordura do leite foi maior (P<0,052) em A (32,02 para 23,93g/100g AG) do que em B (30,17 para 25,45g/100g AG). O teor de ácido vacênico (AV) na gordura do leite aumentou (P<0,001) de 5,70 para 13,24g/100g AG em B e de 5,25 para 16,77 em A, resultando em maior teor de AV neste último (P<0,001). O teor basal de ácido rumênico aumentou expressivamente (P<0,001) em B (1,80 para 4,09g/100g AG, +127%) e A (1,60 para 4,61g/100g AG, +187%), não havendo diferenças entre tratamentos (P<0,19). No geral, estes resultados indicam uma melhora pronunciada no valor nutricional da gordura do leite de búfalas a pasto recebendo pequenas quantidades (0,276g/dia) de uma mistura de óleos de soja e linhaça na dieta.

Palavras-chave: búfalos, ácido linoleico conjugado, lactação, óleo de soja, óleo de linhaça

INTRODUCTION

Buffalo milk is a rich source of nutrients with higher levels of total protein, medium chain FA, conjugated linoleic acid (CLA) and contents of retinol and tocopherols than those of cow milk (Ahmad *et al*., 2013). Higher amounts of saturated fatty acids (SFA) and lower amounts of unsaturated fatty acids (UFA) were also reported in buffalo milk compared to cow milk together with higher contents of myristic (14:0) and palmitic (16:0) FA (Ménard *et al.*, 2010). These FA (14:0 and 16:0) are classed as atherogenic (Ulbritch and Southgate, 1991) if consumed in excess and associated to increased risk of heart disease (Stanton *el al.*, 2003; Chilliard and Ferlay, 2004). The supplementation with polyunsaturated rich oils may decrease the concentration of the potentially atherogenic FA in cows (Rego *et al.*, 2005) and in lactating buffaloes (Oliveira *et al.*, 2009). Concerning the potential functional benefits of milk, a current special interest exists on conjugated linoleic acid (CLA) concentration, because it plays an important role in regulating plasma lipid concentrations and cardiovascular functions, reducing cancer incidence, as well as blocking tumor growth and metastasis from cancer breasts (Parodi, 1999). Between the natural CLA isomers, the *cis*- 9 *trans*-11 18:2 or rumenic acid (RA) is the most present (80-90%) in milk fat. Vaccenic acid (VA, *trans*-11 18:1) is the main natural *trans* FA and precursor of RA in the mammary gland and other tissues. It may also have anticarcinogenic properties and can be metabolized by humans to RA (Stanton *et al*., 2003). Buffalo milk contains higher amounts of RA and VA than cow milk (Ahmad *et al*., 2013; Ménard *et al.*, 2010).). Milk fat is considered the main natural source of RA and its concentration in milk is highly dependent on the type of diet and lipid supplementation in dairy cows (Chilliard *et al*., 2007) and buffaloes (Fernandes *et al*., 2007; Patiño *et al.*, 2010, Oliveira *et al.* 2009). To our knowledge the information about the effect of providing a combination of soy and linseed oils on the full milk FA profile of grazing buffaloes is non-existent. The aim of this experiment is to evaluate the effect of supplementation with a blend (70:30 wt/wt) of soybean and linseed oils on the chemical composition of milk and the FA profile in lactating buffaloes in natural grassland.

MATERIALS AND METHODS

The experiment was conducted during the months of October - November 2013 at the commercial farm of Nuestra Señora de Itatí (Route 12, Km 1098, Itatí Department, site Yacareí Province of Corrientes, Argentina) with an 80 hectares field and natural grassland. Fourteen multiparous Mediterranean buffaloes (milked after two years of inactivity) between the second and the third part of their lactation were used (7 with L and 7 with H doses). Experimental procedures and buffalo management were performed according to the recommendations of good management practices (GMP) of the National Sanitarian Service (SENASA, Argentina). As the experiment progressed animals were gradually adapted to the milking routine and oxytocin was used to

facilitate milk release resulting in a gradual increase in daily milk production as the experiment wore on. Animals grazed a native pasture composed by Andropogon lateralis (NDF=64.5%; ADF=39.1%, lignin=5.1% and CP=6.4%), Schyzachirium paniculatum (CP=4-10%) and Paspalum notatum (NDF=59.2%, ADF=35.4%, Lignin=3.5% and CP= 8.5%). They were supplemented with 4 kg/day of a commercial concentrate (87.95% DM; 4.21% EE, 14.52% CP, 65.57% NDF, 11.03% ADF, 89.00% TDN and ED 3.97 Mcal DE/kg DM) composed (DM basis) by cracked corn grain (50%), rice bran (10%), pelleted sunflower (10%), soybean (5%) and wheat meals, bovine fat (11%) and a mineral premix (4%). Ten days prior to the start of the experiment the animals consumed pasture (63%) plus concentrate (27%) without oil to determine the baseline (B) milk composition and FA profile on samples taken over the last three days. After this period the animals were supplemented with a blend (70:30, wt/wt) of soybean and linseed oils at a low (L, 276g/d) and a high (H, 572g/d) doses mixed to the concentrate. Oil represented about 2.21 and 4.42% of total DM intake if a maximum DM intake of 12.5 kg/animal/day is assumed (Kathirvelan and Tyagi, 2009). Oils were manually mixed to the concentrate and thoroughly consumed during milking time in the morning. After milking animals were left in the grazing paddock until the next day.

Animals were milked mechanically once a day at 8am and individual samples (100 ml) were collected. An aliquot of 10mL was used for chemical analysis while the remaining 90mL were immediately frozen (-20) until FA determination by GLC. Before the start of oil supply milk yield was individually recorded and milk samples were obtained during three consecutive days to measure the B milk composition and FA profile. The procedure was repeated over the last 3 days of the experimental period. Milk samples were analyzed for fat, protein and nonfat solids by infrared spectrophotometry (Milk analyzing device Model: Master Eco). The calibration routine is done with reference to a standard pattern using buffalo milk quantified by other methods used values (Fat by the Gerber method; Acidity by the Dornic method; Protein by the Kjeldahl method). Milk FA composition was measured in individual samples from the three days of each period according to guidelines of Bulletin of International Dairy Federation N° 265/1991 (“Determination of free fatty acids in milk and milk products" ISO 15884-IDF 182: Milk fat, preparation of fatty acid methyl esters).

STATISTICAL ANALYSES

Data of milk production and composition and milk FA profile were analyzed using the Student T-test for paired observations. Differences between L and H oil doses were stated using the Student t-test for independent observations (n=7). Differences were considered significant with $P<0.05$ unless otherwise stated. Values are presented as means followed by standard deviation.

RESULTS

Oil content for linoleic (53.61 and 15.90%) and linolenic (0.36 and 50.39%) FA presented normal values for soybean and linseed oils respectively. At the start of the experiment, milk yield (g/animal/day) averaged 1776 (±522) and 1662 (±291) for L and H respectively (Table 1) without differences between treatment groups ($P<0.622$). At the end of the trial milk yield averaged 4590 and 4847g/animal/day in L and H (Table 1) without differences between oil doses ($P<0.543$).

Compared to the basal diet, milk fat content decreased in L and H whereas milk protein content increased after oil supplementation. Concentrations of *de novo* (4:0 a 15:1) and short chain FA (4:0 to 10:0) were reduced only in the H treatment (Table 2). Milk concentration of 12:0 was not affected, but the concentration of 14:0 decreased (-24%, $P<0.007$) only in H (Table 2). Concentrations of 16:0 were reduced ($P<0.001$) in both the L and H treatments (Table 2) without differences between the two doses (Table 2). The hypercholesterolemic fraction of milk fat (12:0 to 16:0) was reduced ($P<0.001$) in both treatments but the resulting decrease was higher ($P<0.052$) in H (-8.09g/100g FA) compared to L (-4.72g/100g FA). The atherogenic index of milk was reduced and was lower in H compared to L ($P<0.056$). The concentration of VA increased ($P<0.001$) in L and H and had a higher result ($P<0.001$) in H (16.77g/100g FA) than in L (13.24g/100g FA). Basal RA sharply increased in L (+127%) and H

(+187%) without differences between treatments (P<0.195). The basal RA/VA ratio did not change after oil supplementation. Basal concentrations of *trans*-10 18:1 were low and increased by the oil-blend without differences between treatments. Basal concentrations of elaidic (*trans*-9 18:1) acid in milk increased after oil supplementation without differences between L and H. The basal n-6/n-3 ratio was slightly increased after oil supplementation with lower results in L compared to H doses.

Table 1. Milk yield and composition in lactation buffaloes before (Basal) and 23 days after (Final) supplementation with a blend (70:30, wt:wt) of soybean and linseed oils at a low (L, 276g/d) and a high (H, 572g/d) dose.

	Basal	Final	P< [(1)]
Low oil supplementation (276g/d)			
Milk yield (g/animal/day)	1776a (± 522)	4590b (± 991)	0.0001
Protein (g/100g)	4.32a (± 0.34)	7.32b (± 0.60)	0.0001
Fat (g/100g)	7.71a (± 2.05)	6.07b (± 2.36)	0.031
Total solids (g/100g)	17.83a (±2.22)	21.89b (± 2.40)	0.0001
High oil supplementation (572g/d)			
Milk yield (g/animal/day)	1662a (± 291)	4847b (± 447)	0.0001
Protein (g/100g)	4.43a (± 0.69)	6.84b (± 0.49)	0.0001
Fat (g/100g)	7.43a (± 0.99)	4.94b (± 1.12)	0.0031
Total solids (g/100g)	17.40a (± 1.59)	20.62b (± 1.13)	0.0011

[(1)] a, b Student T-test for paired observations. Differences between L and H oil doses were not significant (Student T-test for independent observations, n=7).

DISCUSSION

The increase in basal milk production after oil supplementation was probably explained by a gradual adaptation of animals to milking and also by the use of oxytocin to promote the release of milk. No effect of lipid dose on milk production was observed. Supplementation with PUFA-rich oils did not affect milk yield in non- grazing (Gagliostro and Chilliard, 1992) or in grazing experiments (Schroeder *et al*., 2004) with dairy cows. Basal content of total solids in milk (17.6%) was in the normal range (17% to 18.1%) reported by Oliveira *et al.* (2009) using confined buffaloes and Ahmad *et al*. (2013) for their review on general composition of buffalo milk. After oil feeding this parameter increased up to 21.89g/100g probably as the consequence of the higher milk protein content after the intake of oil. The increase in total solids makes this milk ideal for processing into dairy products and may also contribute to significant energy savings in manufacturing the milk. Before oil feeding milk protein content was in the normal range (4.0 to 5.0%) reported by Oliveira *et al.* (2009) and Ahmad *et al.* (2013). After oil intake, this parameter showed a noticeable increase reaching values as high as 6.84 and 7.32%. Milk protein content was not affected when confined buffaloes were supplemented with different lipid sources (Oliveira *et al.*, 2009). As synthesis of milk protein can be limited by energy availability some additional energy absorbed after oil intake could improve energy status of the buffaloes and enhance milk protein synthesis explaining in part the increase in milk protein content. In fact, a possible dilution effect of milk protein was not observed as milk production increased. Average basal values of fat (7.43-7.71%, Table 1) were below the normal concentration (8.3%) reported by Varrichio *et al*. (2007). The lower milk fat content after oil supplementation (L= -1.64g/100g and H= -2.49g/100g, Table 1) could be explained in part by a dilution effect as milk yield increased (Table 1) and was not reported for confined buffaloes supplemented with soybean oil at a rate of 2.21% of total DM intake (Oliveira *et al*., 2009). Fat content in milk (8.66 to 9.40g/100g) and in mozzarella cheese (21.05 to 25.27g/100g) were increased (P<0.05) by supplementation with soybean oil (Oliveira *et al*., 2009). In our trial the lower milk fat concentration could also be explained by the increase (and ulterior transfer to the udder) of some trans-FA formed by ruminal biohydrogenation which have antilipogenic properties (Shingfield *et al*., 2010). The uptake of trans-10, cis-12 CLA, trans-9, cis-11 CLA,

cis-10, trans-12 CLA and trans-8, cis-10 CLA reduce the activity and/or expression of genes that encode important enzymes involved in uptake, synthesis and desaturation of FA in the mammary gland (Chilliard *et al*., 2000, Shingfield *et al*., 2010). The decrease in milk fat content at both oil doses was mainly explained by a reduction in the hypercholesterolemic FA of milk fat improving the health benefits of the product.

Table 2. Milk fatty acid composition in lactation buffaloes before (Basal) and after (Final) supplementation with a blend (70:30 wt/wt) of soybean and linseed oils at low (LO, 276g/d) and a high (HO, 572g/d) doses

	LO			HO		Final LO vs Final HO	
	Basal	Final	$P<$[(1)]	Basal	Final	$P<$[(1)]	$P<$[(2)]
4:0	2.44(± 0.31)	2.69(± 0.48)	0.125	2.50(±0.28)	2.23(±0.21)	0.053	0.036
6:0	0.70(±0.14)	0.79(± 0.27)	0.311	0.71(±0.10)	0.61(±0.13)	0.084	0.130
8:0	0.25(± 0.06)	0.29(± 0.11)	0.272	0.24(± 0.05)	0.21(± .06)	0.193	0.117
10:0	0.46(± 0.10)	0.52(± 0.19)	0.252	0.45(± 0.09)	0.40(± .11)	0.332	0.180
12:0	0.79(± 0.13)	0.86(± 0.20)	0.215	0.80(± 0.11)	0.73(±0.13)	0.111	0.169
14:0	4.88(± 0.70)	4.85(± 0.98)	0.880	5.29(± 0.77)	4.03(±0.41)	0.007	0.067
Iso 15:0	0.73(± 0.08)	0.62(± 0.13)	0.170	0.75(± 0.14)	0.42(±0.13)	0.002	0.016
15:0	1.26(± 0.12)	0.90(± 0.06)	0.001	1.35(± 0.10)	0.83(±0.06)	0.001	0.028
15:1	0.40(± 0.05)	0.24(± 0.02)	0.001	0.39(± 0.06)	0.17(±0.03)	0.001	0.001
16:0	24.49(±0.67)	19.75(±0.77)	0.001	25.92(±2.14)	19.17(±0.89)	0.001	0.211
12:0 +14:0 +16:0	30.17(±1.24)	25.45±1.82)	0.001	32.02(±0.76)	23.93(±0.45)	0.001	0.052
A. Index [(2)]	1.09(± 0.13)	0.83(± 0.15)	0.001	1.24(± 0.18)	0.71(± 0.03)	0.001	0.056
16:1	1.22(± 0.13)	0.87(± 0.86)	0.012	1.23(± 0.27)	0.68(± 0.15)	0.002	0.042
17:0	1.19(± 0.20)	0.66(± 0.05)	0.001	1.13(± 0.12)	0.58(±0.04)	0.001	0.008
17:1	0.41(± 0.08)	0.18(± 0.02)	0.001	0.36(± 0.05)	0.14(± 0.03)	0.001	0.005
18:0	19.35(± .80)	18.16(± .52)	0.297	19.80(±2.97)	19.09(±0.31)	0.513	0.391
trans-8 18:1	0.60(± 0.13)	1.08(± 0.14)	0.002	0.61(± 0.08)	1.09(± 0.08)	0.001	0.956
trans-9 18:1	0.40(± 0.07)	0.74(± 0.07)	0.001	0.38(± 0.02)	0.75(± 0.07)	0.001	0.773
trans-10 18:1	0.44(± 0.20)	0.91(± 0.28)	0.034	0.42(± 0.06)	0.85(± 0.22)	0.003	0.698
trans-11 18:1 (VA)	5.70(± 0.37)	13.24±1.66)	0.001	5.25(± 0.62)	16.77(±1.36)	0.001	0.001
cis-9 18:1	28.90(±0.55)	25.06 ±2.41)	0.020	27.17(±2.55)	23.09(±2.38)	0.001	0.148
cis-11 18:1	0.88 (± 0.07)	0.94 (± 0.07)	0.235	0.90 (± 0.05)	1.01 (± 0.10)	0.050	0.158
trans-9,*trans*-12 18:2	0.08 (± 0.01)	0.16 (± 0.02)	0.001	0.08 (± 0.01)	0.20 (± 0.04)	0.001	0.035
cis-9,*cis*-12 18:2	1.73 (± 0.17)	1.74 (± 0.10)	0.819	1.78 (± 0.19)	1.80 (± 0.27)	0.877	0.668
C18:3 *c*9, *c*12, *c*15	0.64 (± 0.13)	0.49 (± 0.06)	0.043	0.60 (± 0.13)	0.42 (± 0.09)	0.002	0.143
cis-9,*trans*-11 18:2 (RA)	1.80 (± 0.27)	4.09 (± 0.59)	0.001	1.60 (± 0.31)	4.61 (± 0.83)	0.001	0.195
De novo FA (4:0-15:1)	12.00(±1.30)	11.84 (± .31)	0.792	12.59(±1.35)	9.70 (± 0.99)	0.004	0.044
Preformed FA (>17:0)	62.27(±1.58)	67.54(±3.02)	0.001	60.24(±3.39)	70.46(±0.48)	0.001	0.026
RA/VA ratio	0.32 (± 0.04)	0.31 (± 0.05)	0.919	0.31 (± 0.06)	0.28 (± 0.06)	0.333	0.278
Short chain FA (4:0 to 10:0)	3.85 (± 0.59)	4.30 (± 1.03)	0.159	3.90 (± 0.35)	3.45 (± 0.39)	0.086	0.063
Long chain FA (18:0 to 22:6)	60.67(±0.38)	66.70 (± .00)	0.001	58.75(±3.29)	69.75(±0.52)	0.001	0.021
n-6/ n-3	2.46 (± 0.41)	3.32 (± 0.25)	0.004	2.71 (± 0.31)	4.03 (± 0.46)	0.001	0.004

[1]Probability of Student T-test for paired observations. [2]Probability of Student T-test for independent observations (Final LO vs Final HO). [3]Atherogenicity Index = [(12:0 + 414:0 + 16:0)/∑ unsaturated FA)]. Ulbritch and Southgate, 1991.

Concentrations of butyric and linolenic acids resulted in values close to those reviewed by Ahmad et al. (2013), while those of caproic, caprylic, capric, lauric, myristic and palmitic acids were lower and stearic, trans-10 octadecenoic, vaccenic, oleic (cis-9 18:1), linoleic (cis-9, cis-12 18:2) and rumenic acids had higher results. Average concentrations of stearic (107%), oleic (+42%), vaccenic (+185%), rumenic (+100%), total trans 18:1 + rumenic (+98%) and the n-6/n-3 ratio (89%) had higher results, whereas those of lauric (-66%), myristic (-59%) and palmitic (-32%) were lower than those reviewed by Ahmad *et al*. (2013).

Regarding saturated FA, Soliman *et al*. (1979) observed an average total content of 71.7% in Egyptian buffalo milk fat, a value resulted near

to that reported by Oliveira *et al*., (2009) in their confined experiment (69.8%) but higher than the observed in the milk fat (56-59%) for the grazing buffaloes used in the present experiment. Polidori *et al*. (1997) also reported that buffalo milk contains about 67% of saturated FA, while Van Nieuwenhove *et al*. (2004) reported values of 59% for long-chain saturated FA in animals fed on natural pastures. After oil intake total SFA in milk decreased up to 50.09% in L and 48.3% in H doses with a UFA/SFA ratio near to 1.

In Oliveira *et al*. (2009) feeding soybean oil resulted in an approximate 10% decrease in SFA relative to the control treatment or when oil was supplied as soybean grain. In Oliveira *et al*. (2009), baseline for the hypercholesterolemic FA (45.8g/100g) was higher than the average observed in our grazing experiment (30.15 to 32.02g/100g, Table 2). Beyond type of diet, milk concentration of 12:0 and 14:0 was also associated with higher body condition of buffaloes (Ahmad *et al.*, 2013) and the difference with Oliveira's data may also be explained by the moderate body condition of our grazing animals. In the study by Fernandes *et al.* (2007) the hypercholesterolemic FA varied from 32.48 to 42.9%. When considering only the SFA able to raise LDL blood levels (lauric, mirystic and palmitic) feeding the oil-blend has reduced their concentrations with emphasis on H (23.93g/100g) compared to L (25.45g/100g) doses (Table 2). After supplementation with the oil-blend, the concentration of human atherogenic FA was lower than the values of 43g/100g reported by Oliveira *et al*. (2009). Supplementation with the high dose of oil-blend was the most effective way to reduce the total concentration of hypercholesterolemic FA and the atherogenic index of milk fat (Table 2).

Average baseline records for 18:0 (19.57g/100g) were well above the range (7.86-13.44) reported by Fernandes *et al*. (2007) for Brazilian buffaloes. Supplementary 18:2 did not enhance the concentration of 18:0 in milk fat (Table 2) suggesting that increased biohydrogenation of 18:2 (and also VA) in the rumen probably did not occur. Feeding soybean oil but not soybean grain reduced the concentration of 18:0 in milk fat of lactating buffaloes (Oliveira *et al.*, 2009).

In our experiment, average basal content of oleic acid (28.04g/100g) was above the range (20.6 to 25.1g/100g) reported by Fernandes *et al*. (2007) for Brazilian buffaloes resulting also well above than observed in confined buffaloes (20.35g/100g) fed TMR diets (Oliveira *et al.*, 2009) but near the values of 29.47g/100g reported by Qurshi *et al*. (2010). In confined diets (Oliveira *et al.*, 2009), feeding soybean oil (but not soybean grain) increased oleic acid in milk up to 25g/100g, whereas in our experiment this FA decreased in both L and H oil doses (Table 2). As oils contained about 19% of oleic acid (Table 1) a high rumen hydrogenation of this FA seems probable. Some inhibition by *trans* isomers of delta-9 desaturase enzyme in the mammary gland (Shingfield *et al.*, 2010) may also explain the observed reduction in oleic acid. In our trial, average basal concentration of RA (1.70g/100g FA) was in the upper range limit (1.02-1.77g/100g) reported by Fernandes *et al.* (2007) for buffaloes on commercial farms in Brazil although the average values observed were about 1.1g/100g in four of the five tested farms.

Baseline RA (Table 2) was 1.74 times higher than values of 0.98g/100g FA reported by Oliveira *et al.* (2009). The relative increase in milk RA after oil supplementation (127% and 188%) was higher than the obtained by Oliveira *et al.* (2009) in buffaloes supplemented with soybean grain (+13.3%) or soybean oil (+102%). The average RA concentration obtained after oil supplementation (4.35g/100g, Table 2) was higher than the total CLA concentration of 1.95g/100g when 2% of mustard oil was added to the diet of Murrah buffaloes (Kathirvelan and Tyagi, 2009). The relative increase in H oil treatment (188%, Table 2) was near to that informed (185%) by Kathirvelan and Tyagi (2009). In their experiment the average total CLA contents (g/100g milk fat) was 0.684, 1.212 and 1.95 when animals were fed groundnut cake-based concentrate, mustard cake-based concentrate and 2% of mustard oil respectively.

Baseline of total *trans* FA in milk (6.90g/100g, Table 2) was sharply increased (17.72g/100g) after oil supplementation. Intake of *trans* fatty acids has been associated with an increased risk of human cardiovascular disease but results from epidemiological studies have indicated that the intake of rumenic *trans* FA is innocuous or even protects against cardiovascular disease (Jakobsen *et al.*, 2008; Bendsen *et al.*, 2011). In the

industrially produced hydrogenated fats, the elaidic acid and *trans*-10 18:1 are the prevailing *trans*-FA while VA is the major *trans*-FA in milk. In our trial, elaidic acid represented only 5.65% of total *trans* and decreased to 4.24% after oil supplementation whereas *trans*-10 18:1 represented about 6.23% and 5.03%, respectively.

Vaccenic acid represented 79.3% and 84.5% of total *trans* in basal or after oil supplementation respectively (Table 2). At about 2.21% of estimated total DMI the oil mix supplied apparently exceeded the desaturation capacity of the mammary gland to convert VA to RA because in doubling the dose an increase in the concentration of VA was obtained without further increases in the content of RA. The presence of VA in dairy products may also have beneficial health properties through its direct anticarcinogenic properties (Awad *et al*., 1995) or mediated through its endogenous conversion to RA by delta-9 desaturases in human tissues (Parodi, 2003). The conversion of VA to RA has been shown to prevent chemically induced carcinogenesis in rodents (Banni *et al*., 2001). In spite of the increased availability of linoleic and linolenic acids after oil supplementation its incorporation to milk fat was negligible suggesting a moderate efficiency in the transfer of these FA to milk and a high rate of rumen biohydrogenation in buffaloes.

CONCLUSIONS

In the extensive production conditions of the present work based on natural grassland diets, buffalo milk represented a good source of RA. Supplementation with a mixture (70:30) of soy and linseed oils at 2.21% of estimated DM intake reduced milk fat concentration, increased milk protein and total solid contents and induced profound changes in the milk FA profile. The concentration of the potentially human atherogenic FA and the atherogenic index of milk were attenuated while the RA concentration was sharply increased. This fact should contribute to alleviate the dieticians' criticism against dairy products for their ability to raise LDL cholesterol and to improve the health quality of buffalo milk and its image as perceived by the consumer.

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